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Award Number:
W81XWH-08-1-0084

TITLE:
Using Human Stem Cells to Study the Role of the Stroma in the Initiation of Prostate Cancer

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REPORT DATE:
March 2009

TYPE OF REPORT:
Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
24/03/2009	Annual Report	1 Mar 2008 – 28 Feb 2009

4. TITLE AND SUBTITLE : Using Human Stem Cells to Study the Role of the Stroma in the initiation of Prostate Cancer

5a. CONTRACT NUMBER
W81XWH-08-1-0084

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Gail Risbridger, Ph.D.

5d. PROJECT NUMBER
PC073444.01

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Monash University
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
and Materiel Command

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(s)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project aims to determine the role of tumor stroma in prostate cancer biology. To do this, we are using a model of human embryonic stem cell (hESC) differentiation that was established in our laboratory. Using hESC-derived prostatic epithelial cells, we will test whether or not tumor stroma derived from human prostate cancer specimens will induce and initiate carcinogenesis.

Our first task has been to optimize our current protocols of hESC differentiation into prostate. Ideally, we will eliminate the small percentage of hESCs that spontaneously differentiate into non-prostatic structures in tissue grafts in order to work with a pure population of prostatic cells. Work towards this aim is in progress. In the following 6-12 months, we will begin to isolate prostatic stem cells from our hESC-derived tissues, and subsequently initiate experimental studies with human cancer stroma enriched cell populations.

15. SUBJECT TERMS
None provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
				6	Gail Risbridger
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unlimited		**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Introduction

The role of tumor stroma in prostate cancer biology is equivocal. Current dogma suggests that prostate carcinogenesis is a multi-step process involving genetic alterations in the epithelium that drives the progressive transformation of normal human cells into highly malignant derivatives. It is evident that tumor stroma is able to promote progression of tumorigenesis, but whether it also plays a critical role in the initiation of tumor formation is unclear.

Epithelial cells are under the control of the underlying mesenchymal cells during embryogenesis and throughout life; it is therefore our **hypothesis that the prostatic stromal cells have the capacity to initiate carcinogenesis in normal epithelial cells**. In order to address the issue of tumor initiation, we propose to use normal human prostate epithelium generated from human embryonic stem cells in tissue recombination studies with tumor stroma from human prostate cancer patients.

In this project, we propose to use human embryonic stem cells as a source of normal human prostate epithelial cells. Normal human prostate tissue from adult men in the prime of his life is difficult to obtain, and human fetal tissue is of limited availability. We successfully achieved this goal and published the findings in Nature Methods (Taylor, et al., 2006).

Body

Although funds for this project were released in the first half of 2008, we spent several months getting ethics approval for both animal and human experimental procedures. Although existing institutional approvals were in place, we were required to make several amendments to meet the requirements of US Department of Defense legislation and regulations. This was successfully completed and we obtained formal approval from Human Research Protection Office (Office of Research Protections, U.S. Army Medical Research and Materiel Command) on 7th January 2009 and USAMRMC Animal Care & Use Review Office on 31st December 2008.

During 2008, we employed staff on this project, although they were restricted in the experimental procedures they were able to perform due to pending ethics approval. Therefore, work on this project has progressed slowly, although we have established several key experimental techniques to date, including work towards Task 1 as follows:

Task 1 (Aim 1.1): To improve our current method of directing hESC differentiation to obtain genetically normal human prostatic epithelial cells [Years 0-1.5].

a. Culture and maintenance of human embryonic stem cells (hESCs); including routine karyotyping and identification of other pluripotent markers of undifferentiated hESCs.

- Culture of hESCs has been initiated and maintained. We currently have up to 3 hESC lines growing in the laboratory for use in this project. These cell lines are routinely passaged and over a period of time, have been proven to maintain pluripotentiality and stable karyotype profiles. Staff are well trained in culture of hESCs which is vital to the success of this project.

b. Pre-differentiation of hESCs using 100ng/ml activin A in serum free conditions for 5-8 days into endoderm *in vitro*. Confirm endoderm phenotype using immunohistochemistry and FACs analysis.

- This task was to be performed in collaboration with Professor Alan Trounson. Since the project began, Prof. Trounson has left academic research and now heads the Californian Institute Regenerative Medicine, CA, USA. Since his departure, we have established links with scientists in his laboratory to transfer this technology to our site at MIMR. This has been relatively successful, but has taken some time to optimize conditions of pre-differentiation in our hands. We can now reliably and reproducibly produce ~60-80% definitive endoderm from hESCs using activin A. As described in the proposal, we have used dual fluorescent labeling with Sox17 and CXCR4 to identify the purity of our cell populations. Attempts are currently being made to FACs sort out these cells to enrich for more pure populations that will limit the spontaneous differentiation of hESCs when we use them in tissue recombination assays.

c. Generation of tissue recombinants of endoderm-derived hESCs together with rodent UGM or SVM (isolated from E17.5 male embryos for UGM or day 0 male pups for SVM) using collagen gel technique and sub-renal grafting into male SCID mice.

- We recently initiated tissue recombination studies, following ethics approval that was granted in December 2008. This is an established protocol that is routinely performed in our laboratory. At present, we have set up 2 series of tissue recombination experiments that utilized undifferentiated hESCs and pre-differentiated hESCs. The grafts are presently growing in host male mice and tissues are scheduled to be harvested in April 2009.

d. Harvesting and analysis of tissue recombinants including immunohistochemistry for morphological analysis and cell death/proliferation markers.

- No progress to date.

e. ALTERNATIVE METHOD: perform two-step tissue recombination with endoderm-derived hESCs and rodent UGM or SVM using collagen gel technique and sub-renal grafting into male SCID mice, if first method is not optimal.

- No progress to date.

Key Research Accomplishments:

- Establishment and maintenance of hESC cultures.
- Performed pre-differentiation of hESCs into definitive endoderm.
- Initiate tissue recombination studies with undifferentiated and pre-differentiated hESCs (yet to be analysed).

Reportable Outcomes:

- **Manuscripts:**

1. Taylor RA, Risbridger GP (2008) The path towards identifying prostatic stem cells. **Differentiation** 76(6):671-681 ***(IF 3.745) – JRank 53/156 Cell Biology***

2. Taylor RA, Risbridger GP (2008) Prostatic tumour stroma: a key player in cancer progression. **Current Cancer Drug Targets** 8(6):490-7 ***(IF 5.677) JRank 17/127 Oncology***
3. Risbridger GP, Taylor RA (2008) Prostatic stem cell niche in health and disease. **Endocrinology** 149(9):4303-4306 ***(IF 5.236) – JRank 13/93 Endocrinology & Metabolism***

- **Abstract presentations:**

1. Taylor RA, Toivanen R, Pedersen J, Collins A, Maitland NJ, Risbridger GP (2008) Altered differentiation of CD133+ prostatic stem cells by carcinoma-associated fibroblasts. *The Role of Cancer Stem Cells in the Initiation and Propagation of Tumorigenesis; Special conference of American Association for Cancer Research*, Los Angeles, USA (*poster presentation*).

Conclusion:

In summary, work towards task 1 of this project has begun and it is anticipated that we will begin work towards task 2 in the next 2-4 months. Task 2 involves the isolation of prostatic stem cells from our hESC-derived tissues. Recently there have been several publications that will enable us to isolate enriched prostatic stem cell population using newly identified cell surface makers including CD117 (Leong, et al., 2008) or alternative markers such as Trop2 (Goldstein, et al., 2008). These advances will lead to reliable isolation of prostatic stem cells that have proven regenerative capacity with which to test the effects of prostatic tumor stroma.

The long term goal of defining the role of prostatic tumour stroma in the initiation of carcinogenesis will have a great impact on the field of prostate cancer (and other major cancers) leading to fundamental changes in our thinking about cancer therapy. If the outcomes demonstrate that stroma plays a key role in the initial stages of tumorigenesis, further work will be required to define the stromally-derived factors that may become novel target molecules for early stage prostate cancers.

References

Taylor RA, Cowin PA, Cunha GR, Pera M, Trounson AO, Pedersen J and Risbridger GP (2006) Formation of human prostate tissue from embryonic stem cells. Nat Methods 3:179-181

Leong KG, Wang BE, Johnson L and Gao WQ (2008) Generation of a prostate from a single adult stem cell. Nature 456:804-808

Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP and Witte ON (2008) Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. Proc Natl Acad Sci U S A 105:20882-20887

Appendices

N/A